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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K

A2

(11) International Publication Number: WO 00/08045

(43) International Publication Date: 17 February 2000 (17.02.00)

(21) International Application Number: PCT/US99/17886

(22) International Filing Date: 6 August 1999 (06.08.99)

(30) Priority Data:
09/131,263 7 August 1998 (07.08.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

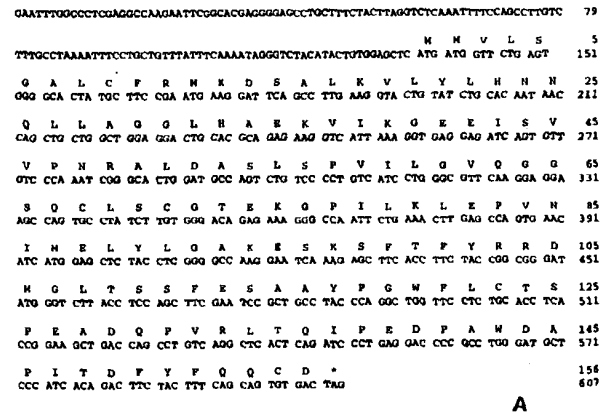
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: NOVEL MOLECULES OF THE TANGO-93-RELATED PROTEIN FAMILY AND USES THEREOF

(57) Abstract

Novel TANGO-93 polypeptides, proteins, and nucleic acid molecules are disclosed. In addition to isolated, full-length TANGO-93 proteins, the invention further provides isolated TANGO-93 fusion proteins, antigenic peptides and anti-TANGO-93 antibodies. The invention also provides TANGO-93 nucleic acid molecules, recombinant expression vectors containing a nucleic acid molecule of the invention, host cells into which the expression vectors have been introduced and non-human transgenic animals in which a TANGO-93 gene has been introduced or disrupted. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.



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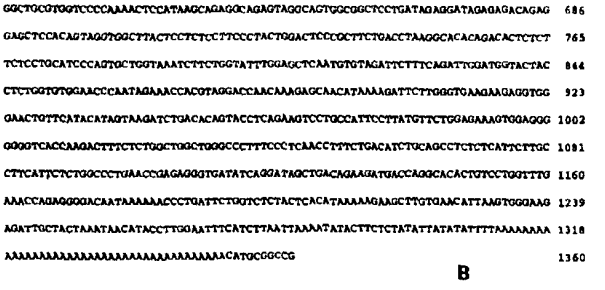

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NOVEL MOLECULES OF THE TANGO-93-RELATED PROTEIN FAMILY AND USES THEREOF

Background of the Invention

Interleukin-1 (IL-1) is a critical mediator of inflammatory and overall immune response. To date, three members of the IL-1 family, IL-1α, IL-1β and IL-1ra (Interleukin-1 receptor antagonist) have been isolated and cloned. IL-1α and IL-1β are proinflammatory cytokines which elicit biological responses, whereas IL-1ra is an antagonist of IL-1α and IL-1β activity. Two distinct cell-surface receptors have been identified for these ligands, the type 1 IL-1 receptor (IL-1RtI) and type II IL-1 receptor (IL-1RtII). Recent results suggest that the IL-1RtI is the receptor responsible for transducing a signal and producing biological effects.

As described above, IL-1 is a key mediator of the host inflammatory response. While inflammation is an important homeostatic mechanism, aberrant inflammation can cause injury or death. Elevated IL-1 levels are known to be associated with a number of disorders, particularly autoimmune and inflammatory disorders. Since IL-1ra is a naturally occurring inhibitor of IL-1, IL-1ra can be used to limit the aberrant and potentially deleterious effects of IL-1. In an animal model of septic shock, pretreatment with IL-1ra has been shown to prevent death resulting from lipopolysaccharide-induced sepsis. The relative absence of IL-1ra has also been suggested to play a role in human inflammatory bowel disease.

Summary of the Invention

The present invention is based, at least in part, on the discovery of genes encoding TANGO-93, a secreted protein that is predicted to be a member of the cytokine superfamily. The TANGO-93 murine cDNA described below (SEQ ID NO:1) is 1360 nucleotides long and includes a 468 nucleotide open reading frame (nucleotides 137-604 of SEQ ID NO:1; SEQ ID NO:3) encoding a 156 amino acid protein (SEQ ID NO:2). The TANGO-93 human cDNA described below (SEQ ID NO:4) is 1323 nucleotides long and includes a 465 nucleotide open reading frame (nucleotides 57-521 of SEQ ID NO:4; SEQ ID NO:6) encoding a 155 amino acid protein (SEQ ID NO:5).

As used herein, the terms "TANGO-93", "TANGO-93 protein", "TANGO-93 polypeptide" refer to any and all TANGO-93 gene products, including those encoded by the cDNA of SEQ ID NO:1 or SEQ ID NO:4, as described above.

TANGO-93 may play a role similar to secreted IL-ra and modulate (e.g., inhibit) inflammation. For example, TANGO-93 may bind to the IL-1 receptor, thus blocking receptor activation by inhibiting the binding of IL-1α and IL-1β to the receptor. Alternatively, Tango-93 may bind an orphan receptor, e.g., IL-18 receptor. IL-18 receptor has significant sequence homology to IL-1 receptor. TANGO-93 may modulate IL-1 gene expression or TANGO-93 may modulate IL-1 protein expression. TANGO-93 may be an inflammatory cytokine itself, regulating immune-mediated inflammation in a manner that is independent of the IL-1 pathway.

The TANGO-93 molecules of the present invention and modulators thereof are potentially useful as modulating agents in regulating a variety of cellular processes such as asthma, graft vs-host disease, rheumatoid arthritis, psoriasis, inflammatory bowel disease, septic shock, ulcerative colitis, Crohn's

disease, chronic myelogenous leukemia, cancer, liver disease, Hodgkin's disease, osteoarthritis, Lyme disease, cachexia, and autoimmune diseases, e.g., myasthenia gravis, autoimmune diabetes, and systemic lupus erthematosus. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding TANGO-93 proteins or biologically active portions thereof, as well as nucleic acid fragments suitable as primers or hybridization probes for the detection of TANGO-93-encoding nucleic acids.

The invention features a nucleic acid molecule which is at least 65% (or 75%, 85%, 95%, or 98%) identical to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the nucleotide sequence of the cDNA insert of the plasmid deposited with ATCC as Accession Number (the "cDNA of ATCC 98820"), or a complement thereof.

The invention features a nucleic acid molecule which includes a fragment of at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, or 1360) nucleotides of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the nucleotide sequence of the cDNA of ATCC 98820, or a complement thereof.

The invention features a nucleic acid molecule which includes a nucleotide sequence encoding a protein having an amino acid sequence that is at least 65% (or 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA of ATCC 98820.

In a preferred embodiment, a TANGO-93 nucleic acid molecule has the nucleotide sequence shown SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the cDNA of ATCC 98820.

Also within the invention is a nucleic acid molecule which encodes a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:2 the fragment including at least 15 (25, 30, 50, 100, 155) contiguous amino acids of SEQ ID NO:2. Also within the invention is a nucleic acid molecule which encodes a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:5 the fragment including at least 15 (25, 30, 50, 100, 156) contiguous amino acids of SEQ ID NO:5, or the polypeptide encoded by the cDNA of ATCC 98820.

The invention includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3 or a complement thereof under stringent conditions.

The invention also includes a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:5, or an amino acid sequence encoded by the cDNA of ATCC 98820, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:4, SEQ ID NO:6 or a complement thereof under stringent conditions.

Also within the invention are: an isolated TANGO-93 protein which is encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 75%, preferably 85%, or 95% identical to SEQ ID NO:3, SEQ ID NO:6, the cDNA of ATCC 98820, and an isolated TANGO-93 protein which is encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:6, its complement, or the non-coding strand of the cDNA of ATCC 98820.

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:2, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3 or a complement thereof under stringent conditions.

Also within the invention is a polypeptide which is a naturally occurring allelic variant of a polypeptide that includes the amino acid sequence of SEQ ID NO:5 or an amino acid sequence encoded by the cDNA of ATCC 98820, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:4, SEQ ID NO:6, the non-coding strand of the cDNA of ATCC 98820 or a complement thereof under stringent conditions.

Another embodiment of the invention features TANGO-93 nucleic acid molecules which specifically detect TANGO-93 nucleic acid molecules relative to nucleic acid molecules encoding other members of the TANGO-93 superfamily. For example, in one embodiment, a TANGO-93 nucleic acid molecule that hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA of ATCC 98820, or a complement thereof. In another embodiment, the TANGO-93 nucleic acid molecule is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, 1200, or 1360) nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA of ATCC 98820, or a complement thereof. In another embodiment, the invention provides an isolated nucleic acid molecule

which is antisense to the coding strand of a TANGO-93 nucleic acid.

Another aspect of the invention provides a vector, e.g., a recombinant expression vector, comprising a TANGO-93 nucleic acid molecule of the invention. In another embodiment the invention provides a host cell containing such a vector. The invention also provides a method for producing TANGO-93 protein by culturing, in a suitable medium, a host cell of the invention containing a recombinant expression vector such that a TANGO-93 protein is produced.

Another aspect of this invention features isolated or recombinant TANGO-93 proteins and polypeptides. Preferred TANGO-93 proteins and polypeptides possess at least one biological activity possessed by naturally occurring human TANGO-93, e.g., (1) the ability to form protein:protein interactions with proteins in the TANGO-93 signalling pathway; (2) the ability to form protein:protein interactions with proteins in the cytokine signalling pathway, (3) the ability to bind TANGO-93 receptor; (4) the ability to bind to an intracellular target; (5) the ability to interact with a protein involved in inflammation; or (6) the ability to bind the IL-1 receptor. Other activities include the ability to induce or suppress the activity or expression of interleukins, cytokines and growth factors.

The TANGO-93 proteins of the present invention, or biologically active portions thereof, can be operably linked to a non-TANGO-93 polypeptide (e.g., heterologous amino acid sequences) to form TANGO-93 fusion proteins. The invention further features antibodies that specifically bind TANGO-93 proteins, such as monoclonal or polyclonal antibodies. In addition, the TANGO-93 proteins or biologically active portions thereof can be

incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides a method for detecting the presence of TANGO-93 activity or expression in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of TANGO-93 activity such that the presence of TANGO-93 activity is detected in the biological sample.

In another aspect, the invention provides a method for modulating TANGO-93 activity comprising contacting a cell with an agent that modulates (inhibits or stimulates) TANGO-93 activity or expression such that TANGO-93 activity or expression in the cell is modulated. In one embodiment, the agent is an antibody that specifically binds to TANGO-93 protein. In another embodiment, the agent modulates expression of TANGO-93 by modulating transcription of a TANGO-93 gene, splicing of a TANGO-93 mRNA, or translation of a TANGO-93 mRNA. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of the TANGO-93 mRNA or the TANGO-93 gene.

In one embodiment, the methods of the present invention are used to treat a subject having a disorder characterized by aberrant TANGO-93 protein activity or nucleic acid expression by administering an agent which is a TANGO-93 modulator to the subject. In one embodiment, the TANGO-93 modulator is a TANGO-93 protein. In another embodiment, the TANGO-93 modulator is a TANGO-93 nucleic acid molecule. In other embodiments, the TANGO-93 modulator is a peptide, peptidomimetic, or other small molecule. In a preferred embodiment, the disorder characterized by aberrant TANGO-93 protein or nucleic acid expression is chronic and acute inflammation.

The present invention also provides a diagnostic assay for identifying the presence or absence of a genetic lesion or mutation characterized by at least one of: (i) aberrant modification or mutation of a gene encoding a TANGO-93 protein; (ii) mis-regulation of a gene encoding a TANGO-93 protein; and (iii) aberrant post-translational modification of a TANGO-93 protein, wherein a wild-type form of the gene encodes a protein with a TANGO-93 activity.

In another aspect, the invention provides a method for identifying a compound that binds to or modulates the activity of a TANGO-93 protein. In general, such methods entail measuring a biological activity of a TANGO-93 protein in the presence and absence of a test compound and identifying those compounds which alter the activity of the TANGO-93 protein.

The invention also features methods for identifying a compound which modulates the expression of TANGO-93 by measuring the expression of TANGO-93 in the presence and absence of a compound.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figure 1 depicts the cDNA sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) of mouse TANGO-93. The open reading frame of SEQ ID NO:1 extends from nucleotide 137 to nucleotide 604 (SEQ ID NO:3).

Figure 2 depicts the cDNA sequence (SEQ ID NO:4) and predicted amino acid sequence (SEQ ID NO:5) of human TANGO-93. The open reading frame of SEQ ID NO:4 extends from nucleotide 57 to nucleotide 521 (SEQ ID NO:6).

Figure 3 depicts an alignment of the amino acid sequence of human TANGO-93 (SEQ ID NO:5) and mouse TANGO-93 (SEQ ID NO:2).

Figure 4 depicts an alignment of the amino acid sequence of mouse TANGO-93 (SEQ ID NO:2), human TANGO-93 (SEQ ID NO:5), human IL-1ra (SEQ ID NO:7), and mouse IL-1ra (SEQ ID NO:8).

Figures 5A and 5B depict the cDNA sequence (SEQ ID NO:9) of a human TANGO-93 clone that contains additional 3' untranslated sequence compared to SEQ ID NO:4. The open reading frame of SEQ ID NO:9 extends from nucleotide 63 to nucleotide 527.

Detailed Description of the Invention

The present invention is based on the discovery of cDNA molecule encoding murine TANGO-93 and human TANGO-93, members of the cytokine superfamily. A nucleotide sequence encoding a murine TANGO-93 protein is shown in Figure 1 (SEQ ID NO:1; SEQ ID NO:3 includes the open reading frame only). A predicted amino acid sequence of murine TANGO-93 protein is also shown in Figure 1 (SEQ ID NO: 2). A nucleotide sequence encoding a human TANGO-93 protein is shown in Figure 2 (SEQ ID NO:4; SEQ ID NO:6 includes the open reading frame only). A predicted amino acid sequence of human TANGO-93 protein is also shown in Figure 2 (SEQ ID NO: 5).

The TANGO-93 murine cDNA of Figure 1 (SEQ ID NO:1), which is approximately 1360 nucleotides long including untranslated regions, encodes a protein amino acid having a molecular weight of approximately 17 kDa (excluding post-translational modifications).

The TANGO-93 human cDNA of Figure 2 (SEQ ID NO:4), which is approximately 1323 nucleotides long including untranslated regions, encodes a protein amino acid having a molecular weight of approximately 17 kDa (excluding

post-translational modifications). A plasmid containing a cDNA encoding human TANGO-93 (with the cDNA insert name of human TANGO-93) was deposited with American Type Culture Collection (ATCC), Manassas, Virginia on July 17, 1988 and assigned Accession Number 98820. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. § 112.

Murine TANGO-93 protein (SEQ ID NO:2) has 91% identity to human TANGO-93 protein (SEQ ID NO:5). In addition, murine TANGO-93 protein (SEQ ID NO:2) has 50% identity to human IL-1ra (SEQ ID NO:7) and 52% identity to mouse IL-1ra (SEQ ID NO:8). Human TANGO-93 protein (SEQ ID NO:5) has 53% identity to human IL-1ra (SEQ ID NO:7).

Using *in situ* hybridization, the expression of mouse TANGO-93 was shown to be developmentally regulated. Briefly, on embryonic Day 14.5, no expression of TANGO-93 was observed in any tissue. However, at embryonic day 15.5, low level expression of mouse TANGO-93 was observed at the tip of the nose and in the pancreas. At embryonic day 16.5, as well as in postnatal day 1.5, expression of mouse TANGO-93 was detected in the skin (most likely squamous epithelium). Northern blot analysis also revealed that a 1.4 kB TANGO-93 mRNA transcript was observed at embryonic day 17 in the skin.

In situ hybridization revealed that mouse TANGO-93 mRNA transcript is also constitutively expressed in adult tissue including the esophagus. No expression of the mouse TANGO-93 mRNA transcript was observed in unstimulated brain, eye, heart, thymus, spleen, lung, skeletal, muscle/diaphragm, transverse colon, ascending

colon, descending colon, small intestine, liver, kidney, bladder, or adrenal gland.

Northern analysis revealed that human TANGO-93 is expressed in placenta, uterus, and skeletal muscle.

Similar to other regulators of inflammation, expression of mouse TANGO-93 mRNA was observed following administration of LPS. Using a PCR assay (TAQ-man) TANGO-93 mRNA transcript was observed in the liver, heart, spleen and bone marrow. Little or no transcript was observed in kidney, brain and lung of LPS-treated mice. The expression profile of mouse TANGO-93 is similar to IL-1ra, except that expression of TANGO-93 was observed in fewer tissue than IL-1ra.

Moreover, murine TANGO-93 mRNA expression is upregulated in Th1 cells, but not Th2 cells. This suggests that murine Tango-93 may mediate inflammatory and autoimmune diseases.

Human TANGO-93 is one member of a family of molecules (the "TANGO-93 family") having certain conserved structural and functional features. The term "family" when referring to the protein and nucleic acid molecules of the invention is intended to mean two or more proteins or nucleic acid molecules having a common structural domain and having sufficient amino acid or nucleotide sequence identity as defined herein. Such family members can be naturally occurring and can be from either the same or different species. For example, a family can contain a first protein of human origin and a homologue of that protein of murine origin, as well as a second, distinct protein of human origin and a murine homologue of that protein. Members of a family may also have common functional characteristics.

Preferred TANGO-93 polypeptides of the present invention have an amino acid sequence sufficiently identical to the amino acid sequence of SEQ ID NO:2 or

SEQ ID NO:5. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural sequence and/or common functional activity. For example, amino acid or nucleotide sequences which contain a common structural sequence having about 65% identity, preferably 75% identity, more preferably 85%, 95%, or 98% identity are defined herein as sufficiently identical.

As used interchangeably herein a "TANGO-93 activity", "biological activity of TANGO-93" or "functional activity of TANGO-93", refers to an activity exerted by a TANGO-93 protein, polypeptide or nucleic acid molecule on a TANGO-93 responsive cell as determined *in vivo*, or *in vitro*, according to standard techniques. A TANGO-93 activity can be a direct activity, such as an association with or an enzymatic activity on a second protein or an indirect activity, such as a cellular signaling activity mediated by interaction of the TANGO-93 protein with a second protein. In a preferred embodiment, a TANGO-93 activity includes at least one or more of the following activities: (1) the ability to form protein:protein interactions with proteins in the TANGO-93 signalling pathway; (2) the ability to form protein:protein interactions with proteins in the cytokine signalling pathway, (3) the ability to bind TANGO-93 receptor; (4) the ability to bind to an intracellular target; (5) the ability to interact with a protein involved in inflammation; or (6) the ability to bind the IL-1 receptor.

Accordingly, another embodiment of the invention features isolated TANGO-93 proteins and polypeptides having a TANGO-93 activity.

Various aspects of the invention are described in further detail in the following subsections.

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode TANGO-93 proteins or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify TANGO-93-encoding nucleic acids (e.g., TANGO-93 mRNA) and fragments for use as PCR primers for the amplification or mutation of TANGO-93 nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Preferably, an "isolated" nucleic acid is free of sequences (preferably protein encoding sequences) which naturally flank the nucleic acid (i.e., sequences located at the 5′ and 3′ ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated TANGO-93 nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic

acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA of ATCC 98820, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the cDNA of ATCC 98820, as a hybridization probe, TANGO-93 nucleic acid molecules can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook et al., eds., *Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

A nucleic acid of the invention can be amplified using cDNA, mRNA or genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to TANGO-93 nucleotide sequences can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA of ATCC 98820, or a portion thereof. A nucleic acid molecule which is

complementary to a given nucleotide sequence is one which is sufficiently complementary to the given nucleotide sequence that it can hybridize to the given nucleotide sequence thereby forming a stable duplex.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding TANGO-93, for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of TANGO-93. The nucleotide sequence determined from the cloning of the human TANGO-93 gene allows for the generation of probes and primers designed for use in identifying and/or cloning TANGO-93 homologues in other cell types, e.g., from other tissues, as well as TANGO-93 homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 consecutive nucleotides of the sense or anti-sense sequence of SEQ ID NO:1, SEQ ID NO:3; of a naturally occurring mutant of SEQ ID NO:1, SEQ ID NO:3. Alternatively, the oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 consecutive nucleotides of the sense or anti-sense sequence of SEQ ID NO:4, SEQ ID NO:6; or the cDNA of ATCC 98820 or of a naturally occurring mutant of SEQ ID NO:4, SEQ ID NO:6, or the cDNA of ATCC 98820.

Probes based on the human TANGO-93 nucleotide sequence can be used to detect transcripts or genomic sequences encoding the same or identical proteins. The probe comprises a label group attached thereto, e.g., a

radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as part of a diagnostic test kit for identifying cells or tissues which mis-express a TANGO-93 protein, such as by measuring levels of a TANGO-93-encoding nucleic acid in a sample of cells from a subject, e.g., detecting TANGO-93 mRNA levels or determining whether a genomic TANGO-93 gene has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion of TANGO-93" can be prepared by isolating a portion of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the cDNA of ATCC 98820, which encodes a polypeptide having a TANGO-93 biological activity, expressing the encoded portion of TANGO-93 protein (e.g., by recombinant expression *in vitro*) and assessing the activity of the encoded portion of TANGO-93.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the cDNA of ATCC 98820, due to degeneracy of the genetic code and thus encode the same TANGO-93 protein as that encoded by the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the cDNA of ATCC 98820.

In addition to the human TANGO-93 nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the cDNA of ATCC 98820, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of TANGO-93 may exist within a population (e.g., the human population). Such genetic polymorphism in the TANGO-93 gene may exist among individuals within a population due to natural allelic

variation. An allele is one of a group of genes which occur alternatively at a given genetic locus. As used herein, the phrase "allelic variant" refers to a nucleotide sequence which occurs at a TANGO-93 locus or to a polypeptide encoded by the nucleotide sequence. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a TANGO-93 protein, preferably a mammalian TANGO-93 protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the TANGO-93 gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations in TANGO-93 that are the result of natural allelic variation and that do not alter the functional activity of TANGO-93 are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding TANGO-93 proteins from other species (TANGO-93 homologues), which have a nucleotide sequence which differs from that of a human TANGO-93, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the TANGO-93 cDNA of the invention can be isolated based on their identity to the human TANGO-93 nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human TANGO-93 cDNA can be isolated based on its identity to human membrane-associated TANGO-93.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, 1000, or 1360) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA of ATCC 98820, or a complement thereof.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA of ATCC 98820, or the complement thereof, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of the TANGO-93 sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID

NO:4, SEQ ID NO:6, or the cDNA of ATCC 98820, thereby leading to changes in the amino acid sequence of the encoded TANGO-93 protein, without altering the biological activity of the TANGO-93 protein. For example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of TANGO-93 (e.g., the sequence of SEQ ID NO:2 or SEQ ID NO:5) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are not conserved or only semi-conserved among TANGO-93 of various species may be non-essential for activity and thus would be likely targets for alteration. Alternatively, amino acid residues that are conserved among the TANGO-93 proteins of various species may be essential for activity and thus would not be likely targets for alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding TANGO-93 proteins that contain changes in amino acid residues that are not essential for activity. Such TANGO-93 proteins differ in amino acid sequence from SEQ ID NO:2 or SEQ ID NO:5, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 55% identical, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5.

An isolated nucleic acid molecule encoding a TANGO-93 protein having a sequence which differs from that of SEQ ID NO:2 or SEQ ID NO:5 can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or

the cDNA of ATCC 98820, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in TANGO-93 is preferably replaced with another amino acid residue from the same side chain family. Alternatively, mutations can be introduced randomly along all or part of a TANGO-93 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for TANGO-93 biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant TANGO-93 protein can be assayed for: (1) the ability to form protein:protein interactions with proteins in the TANGO-93 signalling pathway; (2) the ability to form

protein:protein interactions with proteins in the cytokine signalling pathway, (3) the ability to bind TANGO-93 receptor; (4) the ability to bind to an intracellular target; (5) the ability to interact with a protein involved in inflammation; or (6) the ability to bind the IL-1 receptor. Other activities include the ability to induce and suppress the activity of interleukins, cytokines and growth factors.

The present invention encompasses antisense nucleic acid molecules, i.e., molecules which are complementary to a sense nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire TANGO-93 coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to a noncoding region of the coding strand of a nucleotide sequence encoding TANGO-93. The noncoding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences which flank the coding region and are not translated into amino acids.

Given the coding strand sequences encoding TANGO-93 disclosed herein (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of TANGO-93 mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of TANGO-93 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation

start site of the mouse TANGO-93 mRNA, e.g., an oligonucleotide having the sequence TCGAGTACTACCAA (SEQ ID NO:9) or CACCTCGAGTACTACCC (SEQ ID NO:10), or the antisense oligonucleotide can be complementary to the region surrounding the translation start site of the human TANGO-93 mRNA, e.g., an oligonucleotide having the sequence CGAGGTCTACCAGGAC (SEQ ID NO:11) or GGTCTACCAGGACTCA (SEQ ID NO:12). An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-

methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a TANGO-93 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also

be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

An antisense nucleic acid molecule of the invention can be an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

The invention also encompasses ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave TANGO-93 mRNA transcripts to thereby inhibit translation of TANGO-93 mRNA. A ribozyme having specificity for a TANGO-93-encoding nucleic acid can be designed based upon the nucleotide sequence of a TANGO-93 cDNA disclosed herein (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a TANGO-93-encoding mRNA. *See, e.g.*, Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742.

Alternatively, TANGO-93 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. *See, e.g.*, Bartel and Szostak (1993) *Science* 261:1411-1418.

The invention also encompasses nucleic acid molecules which form triple helical structures. For example, TANGO-93 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the TANGO-93 (e.g., the TANGO-93 promoter and/or enhancers) to form triple helical structures that prevent transcription of the TANGO-93 gene in target cells. *See generally* Helene (1991) *Anticancer Drug Des.* 6(6):569-84; Helene (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14(12):807-15.

In preferred embodiments, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (*see* Hyrup et al. (1996) *Bioorganic & Medicinal Chemistry* 4(1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996), *supra*; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 14670-675.

PNAs of TANGO-93 can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs of TANGO-93 can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (Hyrup (1996), *supra*; or as probes or primers for DNA sequence and hybridization (Hyrup (1996), *supra*; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 14670-675).

In another embodiment, PNAs of TANGO-93 can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of TANGO-93 can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNAse H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996), *supra*). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996), *supra*, and Finn et al. (1996) *Nucleic Acids Res.* 24(17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds such as 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite can be used as a link between the PNA and the 5' end of

DNA (Mag et al. (1989) *Nucleic Acids Res.* 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn et al. (1996) *Nucleic Acids Res.* 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser et al. (1975) *Bioorganic Med. Chem. Lett.* 5:1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (*see, e.g.*, Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W0 88/09810) or the blood-brain barrier (*see, e.g.*, PCT Publication No. W0 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (*see, e.g.*, Krol et al. (1988) *Bio/Techniques* 6:958-976) or intercalating agents (*see, e.g.*, Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

II. Isolated TANGO-93 Proteins and Anti-TANGO-93 Antibodies

One aspect of the invention pertains to isolated TANGO-93 proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise anti-TANGO-93 antibodies. In one embodiment, native TANGO-93 proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, TANGO-93 proteins are

produced by recombinant DNA techniques. Alternative to recombinant expression, a TANGO-93 protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the TANGO-93 protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of TANGO-93 protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, TANGO-93 protein that is substantially free of cellular material includes preparations of TANGO-93 protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of non-TANGO-93 protein (also referred to herein as a "contaminating protein"). When the TANGO-93 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When TANGO-93 protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of TANGO-93 protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or non-TANGO-93 chemicals.

Biologically active portions of a TANGO-93 protein include peptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the TANGO-93 protein (e.g., the amino acid

sequence shown in SEQ ID NO:2 or SEQ ID NO:5), which include fewer amino acids than the full length TANGO-93 proteins, and exhibit at least one activity of a TANGO-93 protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the TANGO-93 protein. A biologically active portion of a TANGO-93 protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native TANGO-93 protein.

Preferred TANGO-93 protein has the amino acid sequence shown of SEQ ID NO:2 or SEQ ID NO:5. Other useful TANGO-93 proteins are substantially identical to SEQ ID NO:2 or SEQ ID NO:5 and retain the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:5 yet differ in amino acid sequence due to natural allelic variation or mutagenesis. Accordingly, a useful TANGO-93 protein is a protein which includes an amino acid sequence at least about 45%, preferably 55%, 65%, 75%, 85%, 95%, or 99% identical to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5 and retains the functional activity of the TANGO-93 proteins of SEQ ID NO:2 or SEQ ID NO:5. In a preferred embodiment, the TANGO-93 protein retains a functional activity of the TANGO-93 protein of SEQ ID NO:2 or SEQ ID NO:5.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or

nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100). Preferably, the two sequences are the same length.

The determination of percent homology between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to TANGO-93 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to TANGO-93 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov. Another

preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The invention also provides TANGO-93 chimeric or fusion proteins. As used herein, a TANGO-93 "chimeric protein" or "fusion protein" comprises a TANGO-93 polypeptide operably linked to a non-TANGO-93 polypeptide. A "TANGO-93 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to TANGO-93, whereas a "non-TANGO-93 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially identical to the TANGO-93 protein, e.g., a protein which is different from the TANGO-93 protein and which is derived from the same or a different organism. Within a TANGO-93 fusion protein the TANGO-93 polypeptide can correspond to all or a portion of a TANGO-93 protein, preferably at least one biologically active portion of a TANGO-93 protein. Within the fusion protein, the term "operably linked" is intended to indicate that the TANGO-93 polypeptide and the non-TANGO-93 polypeptide are fused in-frame to each other. The non-TANGO-93 polypeptide can be fused to the N-terminus or C-terminus of the TANGO-93 polypeptide.

One useful fusion protein is a GST-TANGO-93 fusion protein in which the TANGO-93 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant TANGO-93.

In yet another embodiment, the fusion protein is an TANGO-93-immunoglobulin fusion protein in which all or part of TANGO-93 is fused to sequences derived from a member of the immunoglobulin protein family. The TANGO-93-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a TANGO-93 ligand and a TANGO-93 protein on the surface of a cell, to thereby suppress TANGO-93-mediated signal transduction *in vivo*. The TANGO-93-immunoglobulin fusion proteins can be used to affect the bioavailability of a TANGO-93 cognate ligand. Inhibition of the TANGO-93 ligand/TANGO-93 interaction may be useful therapeutically, both for treating proliferative and differentiative disorders and for modulating (e.g. promoting or inhibiting) cell survival. Moreover, the TANGO-93-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-TANGO-93 antibodies in a subject, to purify TANGO-93 ligands and in screening assays to identify molecules which inhibit the interaction of TANGO-93 with a TANGO-93 ligand.

Preferably, a TANGO-93 chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another

embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (*see, e.g.*, Ausubel et al., *supra*). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). An TANGO-93-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the TANGO-93 protein.

The present invention also pertains to variants of the TANGO-93 proteins (i.e., proteins having a sequence which differs from that of the TANGO-93 amino acid sequence). Such variants can function as either TANGO-93 agonists (mimetics) or as TANGO-93 antagonists. Variants of the TANGO-93 protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the TANGO-93 protein. An agonist of the TANGO-93 protein can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the TANGO-93 protein. An antagonist of the TANGO-93 protein can inhibit one or more of the activities of the naturally occurring form of the TANGO-93 protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the TANGO-93 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the TANGO-93 proteins.

Variants of the TANGO-93 protein which function as either TANGO-93 agonists (mimetics) or as TANGO-93 antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the TANGO-93 protein for TANGO-93 protein agonist or antagonist activity. In one embodiment, a variegated library of TANGO-93 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of TANGO-93 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential TANGO-93 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of TANGO-93 sequences therein. There are a variety of methods which can be used to produce libraries of potential TANGO-93 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential TANGO-93 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (*see, e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of the TANGO-93 protein coding sequence can be used to generate a variegated population of TANGO-93 fragments for screening and subsequent selection of variants of a TANGO-93 protein. In one embodiment, a library of coding sequence

fragments can be generated by treating a double stranded PCR fragment of a TANGO-93 coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the TANGO-93 protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TANGO-93 proteins. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify TANGO-93 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

An isolated TANGO-93 protein, or a portion or fragment thereof, can be used as an immunogen to generate

antibodies that bind TANGO-93 using standard techniques for polyclonal and monoclonal antibody preparation. The full-length TANGO-93 protein can be used or, alternatively, the invention provides antigenic peptide fragments of TANGO-93 for use as immunogens. The antigenic peptide of TANGO-93 comprises at least 8 (preferably 10, 15, 20, or 30) amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 and encompasses an epitope of TANGO-93 such that an antibody raised against the peptide forms a specific immune complex with TANGO-93. Preferred epitopes encompassed by the antigenic peptide are regions of TANGO-93 that are located on the surface of the protein, e.g., hydrophilic regions.

A TANGO-93 immunogen typically is used to prepare antibodies by immunizing a suitable subject (e.g., rabbit, goat, mouse or other mammal) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed TANGO-93 protein or a chemically synthesized TANGO-93 polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic TANGO-93 preparation induces a polyclonal anti-TANGO-93 antibody response.

Accordingly, another aspect of the invention pertains to anti-TANGO-93 antibodies. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds an antigen, such as TANGO-93. A molecule which specifically binds to TANGO-93 is a molecule which binds TANGO-93, but does not substantially bind other molecules in a sample, e.g., a biological sample, which naturally contains TANGO-93.

Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind TANGO-93. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of TANGO-93. A monoclonal antibody composition thus typically displays a single binding affinity for a particular TANGO-93 protein with which it immunoreacts.

Polyclonal anti-TANGO-93 antibodies can be prepared as described above by immunizing a suitable subject with a TANGO-93 immunogen. The anti-TANGO-93 antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized TANGO-93. If desired, the antibody molecules directed against TANGO-93 can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the anti-TANGO-93 antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) *Immunol. Today* 4:72), the EBV-hybridoma technique (Cole et al. (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (*see generally Current*

Protocols in Immunology (1994) Coligan et al. (eds.) John Wiley & Sons, Inc., New York, NY). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with a TANGO-93 immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds TANGO-93.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-TANGO-93 monoclonal antibody (*see, e.g., Current Protocols in Immunology, supra;* Galfre et al. (1977) *Nature* 266:55052; R.H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); and Lerner (1981) *Yale J. Biol. Med.*, 54:387-402. Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line, e.g., a myeloma cell line that is sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused

myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind TANGO-93, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-TANGO-93 antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with TANGO-93 to thereby isolate immunoglobulin library members that bind TANGO-93. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J.* 12:725-734.

Additionally, recombinant anti-TANGO-93 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be

produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al. (1988) *Science* 240:1041-1043; Liu et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu et al. (1987) *J. Immunol.* 139:3521-3526; Sun et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura et al. (1987) *Canc. Res.* 47:999-1005; Wood et al. (1985) *Nature* 314:446-449; and Shaw et al. (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison (1985) *Science* 229:1202-1207; Oi et al. (1986) *Bio/Techniques* 4:214; U.S. Patent 5,225,539; Jones et al. (1986) *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al. (1988) *J. Immunol.* 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of TANGO-93. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion

of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope.

First, a non-human monoclonal antibody which binds a selected antigen (epitope), e.g., an antibody which inhibits TANGO-93 activity, is identified. The heavy chain and the light chain of the non-human antibody are cloned and used to create phage display Fab fragments. For example, the heavy chain gene can be cloned into a plasmid vector so that the heavy chain can be secreted from bacteria. The light chain gene can be cloned into a phage coat protein gene so that the light chain can be expressed on the surface of phage. A repertoire (random collection) of human light chains fused to phage is used to infect the bacteria which express the non-human heavy chain. The resulting progeny phage display hybrid antibodies (human light chain/non-human heavy chain). The selected antigen is used in a panning screen to select phage which bind the selected antigen. Several rounds of selection may be required to identify such phage. Next, human light chain genes are isolated from the selected phage which bind the selected antigen. These selected human light chain genes are then used to guide the selection of human heavy chain genes as follows. The

selected human light chain genes are inserted into vectors for expression by bacteria. Bacteria expressing the selected human light chains are infected with a repertoire of human heavy chains fused to phage. The resulting progeny phage display human antibodies (human light chain/human heavy chain).

Next, the selected antigen is used in a panning screen to select phage which bind the selected antigen. The phage selected in this step display a completely human antibody which recognizes the same epitope recognized by the original selected, non-human monoclonal antibody. The genes encoding both the heavy and light chains are readily isolated and can be further manipulated for production of human antibody. This technology is described by Jespers et al. (1994, *Bio/technology* 12:899-903).

An anti-TANGO-93 antibody (e.g., monoclonal antibody) can be used to isolate TANGO-93 by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-TANGO-93 antibody can facilitate the purification of natural TANGO-93 from cells and of recombinantly produced TANGO-93 expressed in host cells. Moreover, an anti-TANGO-93 antibody can be used to detect TANGO-93 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the TANGO-93 protein. Anti-TANGO-93 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of

suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding TANGO-93 (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors, expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and

adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., TANGO-93 proteins, mutant forms of TANGO-93, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of TANGO-93 in prokaryotic or eukaryotic cells, e.g., bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *supra*. Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *Gene Expression*

Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada et al. (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the TANGO-93 expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerivisae* include pYepSec1 (Baldari et al. (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz et al. (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and pPicZ (InVitrogen Corp, San Diego, CA).

Alternatively, TANGO-93 can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series

(Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook et al., *supra*.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji et al. (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel

and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to TANGO-93 mRNA. Regulatory sequences operably linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub et al. (*Reviews - Trends in Genetics*, Vol. 1(1) 1986).

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact,

be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, TANGO-93 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*supra*), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding TANGO-93 or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) TANGO-93 protein. Accordingly, the invention further provides methods for producing TANGO-93 protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding TANGO-93 has been introduced) in a suitable medium such that TANGO-93 protein is produced. In another embodiment, the method further comprises isolating TANGO-93 from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which TANGO-93-coding sequences have been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous TANGO-93 sequences have been introduced into their genome or homologous recombinant animals in which endogenous TANGO-93 sequences have been altered. Such animals are useful for studying the function and/or activity of TANGO-93 and for identifying and/or evaluating modulators of TANGO-93 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, an "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an

endogenous TANGO-93 gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing TANGO-93-encoding nucleic acid into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. The TANGO-93 cDNA sequence e.g., that of (SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the cDNA of ATCC 98820) can be introduced as a transgene into the genome of a non-human animal. Alternatively, a nonhuman homologue of the human TANGO-93 gene, such as a mouse TANGO-93 gene, can be isolated based on hybridization to the human TANGO-93 cDNA and used as a transgene. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the TANGO-93 transgene to direct expression of TANGO-93 protein to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, U.S. Patent No. 4,873,191 and in Hogan, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the TANGO-93 transgene in its genome and/or expression of TANGO-93 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals

carrying a transgene encoding TANGO-93 can further be bred to other transgenic animals carrying other transgenes.

To create an homologous recombinant animal, a vector is prepared which contains at least a portion of a TANGO-93 gene (e.g., a human or a non-human homolog of the TANGO-93 gene, e.g., a murine TANGO-93 gene) into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the TANGO-93 gene. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous TANGO-93 gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous TANGO-93 gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous TANGO-93 protein). In the homologous recombination vector, the altered portion of the TANGO-93 gene is flanked at its 5' and 3' ends by additional nucleic acid of the TANGO-93 gene to allow for homologous recombination to occur between the exogenous TANGO-93 gene carried by the vector and an endogenous TANGO-93 gene in an embryonic stem cell. The additional flanking TANGO-93 nucleic acid is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (*see, e.g.*, Thomas and Capecchi (1987) *Cell* 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced TANGO-93 gene has homologously recombined with the endogenous TANGO-93 gene are selected (*see, e.g.*, Li et al. (1992) *Cell* 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form

aggregation chimeras (*see, e.g.*, Bradley in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) *Current Opinion in Bio/Technology* 2:823-829 and in PCT Publication Nos. WO 90/11354, WO 91/01140, WO 92/0968, and WO 93/04169.

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the *cre/loxP* recombinase system of bacteriophage P1. For a description of the *cre/loxP* recombinase system, *see, e.g.*, Lakso et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of *Saccharomyces cerevisiae* (O'Gorman et al. (1991) *Science* 251:1351-1355. If a *cre/loxP* recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the *Cre* recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut et al. (1997) *Nature* 385:810-813 and PCT Publication Nos. WO 97/07668 and WO 97/07669. In brief, a cell, e.g., a somatic cell, from the transgenic

animal can be isolated and induced to exit the growth cycle and enter G_o phase. The quiescent cell can then be fused, e.g., through the use of electrical pulses, to an enucleated oocyte from an animal of the same species from which the quiescent cell is isolated. The reconstructed oocyte is then cultured such that it develops to morula or blastocyte and then transferred to pseudopregnant female foster animal. The offspring borne of this female foster animal will be a clone of the animal from which the cell, e.g., the somatic cell, is isolated.

In therapeutic applications anti-TANGO-93 antibodies, like other therapeutic antibodies, are administered parenterally, preferably intravenously or intramuscularly monthly, biweekly, weekly, or more frequently. The preferred dosage is 1-100 mg/kg of body weight, preferably 10-20 mg/kg of body weight. Higher dosages are preferred if the antibody is to be effective within the brain, the preferred dosage for treatment of a particular disorder can be based on results observed with other therapeutic antibodies or it can be determined by one skilled based on testing in animal models. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Lipidation can be used to stabilize antibodies. A method for lipidation is described by Cruikshank et al. (*J. Acquired Immune Deficiency Syndromes and Human Retrovirlolgy*, 14:193-203, 1997).

IV. Pharmaceutical Compositions

The TANGO-93 nucleic acid molecules, TANGO-93 proteins, and anti-TANGO-93 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the

nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water

soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a TANGO-93 protein or anti-TANGO-93 antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a

sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal

administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each

unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. Patent 5,328,470) or by stereotactic injection (*see, e.g.*, Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology); c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and d) methods of treatment (e.g., therapeutic and prophylactic). A TANGO-93 protein

interacts with other cellular proteins and can thus be used for (i) regulation of cellular proliferation; (ii) regulation of cellular differentiation; and (iii) regulation of cell survival. The isolated nucleic acid molecules of the invention can be used to express TANGO-93 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect TANGO-93 mRNA (e.g., in a biological sample) or a genetic lesion in a TANGO-93 gene, and to modulate TANGO-93 activity. In addition, the TANGO-93 proteins can be used to screen drugs or compounds which modulate the TANGO-93 activity or expression as well as to treat disorders characterized by insufficient or excessive production of TANGO-93 protein or production of TANGO-93 protein forms which have decreased or aberrant activity compared to TANGO-93 wild type protein. In addition, the anti-TANGO-93 antibodies of the invention can be used to detect and isolate TANGO-93 proteins and modulate TANGO-93 activity.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

A. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to TANGO-93 proteins or have a stimulatory or inhibitory effect on, for example, TANGO-93 expression or TANGO-93 activity.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-associated form of a TANGO-93 protein or polypeptide or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous

approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al. (1994). *J. Med. Chem.* 37:2678; Cho et al. (1993) *Science* 261:1303; Carrell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al. (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop et al. (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Bio/Techniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or phage (Scott and Smith (1990) *Science* 249:386-390; Devlin (1990) *Science* 249:404-406; Cwirla et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici (1991) *J. Mol. Biol.* 222:301-310).

In one embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-associated form of TANGO-93 protein, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of

the TANGO-93 protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of TANGO-93 or a biologically active portion thereof can be accomplished, for example, by determining the ability of the TANGO-93 protein to bind to or interact with a TANGO-93 target molecule. As used herein, a "target molecule" is a molecule with which a TANGO-93 protein binds or interacts in nature, for example, a molecule on the surface of a cell which expresses a TANGO-93 protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A TANGO-93 target molecule can be a non-TANGO-93 molecule or a TANGO-93 protein or polypeptide of the present invention. In one embodiment, a TANGO-93 target molecule is a component of a signal transduction pathway which facilitates transduction of an extracellular signal (e.g., a signal generated by binding of TANGO-93 molecule to the cell membrane) through the cell membrane and into the cell.

Determining the ability of the TANGO-93 protein to bind to or interact with a TANGO-93 target molecule can be accomplished by one of the methods described above for determining direct binding. In a preferred embodiment, determining the ability of the TANGO-93 protein to bind to or interact with a TANGO-93 target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., a TANGO-93-responsive regulatory element operably linked to a nucleic acid encoding a detectable marker, e.g. luciferase), or detecting a

cellular response, for example, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a TANGO-93 protein or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the TANGO-93 protein or biologically active portion thereof. Binding of the test compound to the TANGO-93 protein can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the TANGO-93 protein or biologically active portion thereof with a known compound which binds TANGO-93 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a TANGO-93 protein, wherein determining the ability of the test compound to interact with a TANGO-93 protein comprises determining the ability of the test compound to preferentially bind to TANGO-93 or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting TANGO-93 protein or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the TANGO-93 protein or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of TANGO-93 can be accomplished, for example, by determining the ability of the TANGO-93 protein to bind to a TANGO-93 target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of TANGO-93 can be accomplished by determining the ability of the TANGO-93 protein to further modulate a TANGO-93 target molecule.

For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting the TANGO-93 protein or biologically active portion thereof with a known compound which binds TANGO-93 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a TANGO-93 protein, wherein determining the ability of the test compound to interact with a TANGO-93 protein comprises determining the ability of the TANGO-93 protein to preferentially bind to or modulate the activity of a TANGO-93 target molecule.

The cell-free assays of the present invention are amenable to use of both the soluble form or the membrane-associated form of TANGO-93. In the case of cell-free assays comprising the membrane-associated form of TANGO-93, it may be desirable to utilize a solubilizing agent such that the membrane-associated form of TANGO-93 is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton X-100, Triton X-114, Thesit, Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either TANGO-93 or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to TANGO-93, or interaction of TANGO-93 with a target

molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/TANGO-93 fusion proteins or glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or TANGO-93 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of TANGO-93 binding or activity determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either TANGO-93 or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated TANGO-93 or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with TANGO-93 or target molecules but which do not interfere with binding of the TANGO-93 protein to its target molecule can be derivatized to the wells of the plate, and unbound target or TANGO-93 trapped in the wells

by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the TANGO-93 or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the TANGO-93 or target molecule.

In another embodiment, modulators of TANGO-93 expression are identified in a method in which a cell is contacted with a candidate compound and the expression of TANGO-93 mRNA or protein in the cell is determined. The level of expression of TANGO-93 mRNA or protein in the presence of the candidate compound is compared to the level of expression of TANGO-93 mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of TANGO-93 expression based on this comparison. For example, when expression of TANGO-93 mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of TANGO-93 mRNA or protein expression. Alternatively, when expression of TANGO-93 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of TANGO-93 mRNA or protein expression. The level of TANGO-93 mRNA or protein expression in the cells can be determined by methods described herein for detecting TANGO-93 mRNA or protein.

In yet another aspect of the invention, the TANGO-93 proteins can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (*see, e.g.*, U.S. Patent No. 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J. Biol. Chem.* 268:12046-12054; Bartel et al. (1993) *Bio/Techniques* 14:920-924; Iwabuchi et al. (1993) *Oncogene* 8:1693-1696; and PCT Publication No. WO

94/10300), to identify other proteins, which bind to or interact with TANGO-93 ("TANGO-93-binding proteins" or "TANGO-93-bp") and modulate TANGO-93 activity. Such TANGO-93-binding proteins are also likely to be involved in the propagation of signals by the TANGO-93 proteins as, for example, upstream or downstream elements of the TANGO-93 pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for TANGO-93 is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming an TANGO-93-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with TANGO-93.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene

sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. Accordingly, TANGO-93 nucleic acid molecules described herein or fragments thereof, can be used to map the location of TANGO-93 genes on a chromosome. The mapping of the TANGO-93 sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, TANGO-93 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the TANGO-93 sequences. Computer analysis of TANGO-93 sequences can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the TANGO-93 sequences will yield an amplified fragment.

Somatic cell hybrids are prepared by fusing somatic cells from different mammals (e.g., human and mouse cells). As hybrids of human and mouse cells grow and divide, they gradually lose human chromosomes in random order, but retain the mouse chromosomes. By using media in which mouse cells cannot grow (because they lack a particular enzyme), but in which human cells can, the one

human chromosome that contains the gene encoding the needed enzyme will be retained. By using various media, panels of hybrid cell lines can be established. Each cell line in a panel contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, allowing easy mapping of individual genes to specific human chromosomes. (D'Eustachio et al. (1983) *Science* 220:919-924). Somatic cell hybrids containing only fragments of human chromosomes can also be produced by using human chromosomes with translocations and deletions.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the TANGO-93 sequences to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a TANGO-93 sequence to its chromosome include *in situ* hybridization (described in Fan et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. Chromosome spreads can be made using cells whose division has been blocked in metaphase by a chemical, e.g., colcemid, that disrupts the mitotic spindle. The chromosomes can be treated briefly with trypsin, and then stained with Giemsa. A pattern of light and dark bands develops on each chromosome, so that the chromosomes can be identified individually. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher

likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection. Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma et al., (Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York, 1988)).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, e.g., Egeland et al. (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the TANGO-93 gene can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as

deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The TANGO-93 sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the TANGO-93 sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from

individuals and from tissue. The TANGO-93 sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency of about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or SEQ ID NO:4 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or SEQ ID NO:6 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from TANGO-93 sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial TANGO-93 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to

amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or SEQ ID NO:4 are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the TANGO-93 sequences or portions thereof, e.g., fragments derived from the noncoding regions of SEQ ID NO:1 or SEQ ID NO:4 having a length of at least 20 or 30 bases.

The TANGO-93 sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an *in situ* hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such TANGO-93 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., TANGO-93 primers or probes can be used to screen tissue culture for

contamination (i.e., screen for the presence of a mixture of different types of cells in a culture).

C. Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining TANGO-93 protein and/or nucleic acid expression as well as TANGO-93 activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant TANGO-93 expression or activity. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with TANGO-93 protein, nucleic acid expression or activity. For example, mutations in a TANGO-93 gene can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with TANGO-93 protein, nucleic acid expression or activity.

Another aspect of the invention provides methods for determining TANGO-93 protein, nucleic acid expression or TANGO-93 activity in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual examined to determine the

ability of the individual to respond to a particular agent.)

Yet another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs or other compounds) on the expression or activity of TANGO-93 in clinical trials.

These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of TANGO-93 in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting TANGO-93 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes TANGO-93 protein such that the presence of TANGO-93 is detected in the biological sample. A preferred agent for detecting TANGO-93 mRNA or genomic DNA is a labeled nucleic acid probe capable of hybridizing to TANGO-93 mRNA or genomic DNA. The nucleic acid probe can be, for example, a full-length TANGO-93 nucleic acid, such as the nucleic acid of SEQ ID NO: 1 or 3, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to TANGO-93 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting TANGO-93 protein is an antibody capable of binding to TANGO-93 protein, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by

coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect TANGO-93 mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of TANGO-93 mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of TANGO-93 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of TANGO-93 genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of TANGO-93 protein include introducing into a subject a labeled anti-TANGO-93 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control

subject, contacting the control sample with a compound or agent capable of detecting TANGO-93 protein, mRNA, or genomic DNA, such that the presence of TANGO-93 protein, mRNA or genomic DNA is detected in the biological sample, and comparing the presence of TANGO-93 protein, mRNA or genomic DNA in the control sample with the presence of TANGO-93 protein, mRNA or genomic DNA in the test sample.

The invention also encompasses kits for detecting the presence of TANGO-93 in a biological sample (a test sample). Such kits can be used to determine if a subject is suffering from or is at increased risk of developing a disorder associated with aberrant expression of TANGO-93 (e.g., an immunological disorder). For example, the kit can comprise a labeled compound or agent capable of detecting TANGO-93 protein or mRNA in a biological sample and means for determining the amount of TANGO-93 in the sample (e.g., an anti-TANGO-93 antibody or an oligonucleotide probe which binds to DNA encoding TANGO-93, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4 or SEQ ID NO:6). Kits may also include instruction for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of TANGO-93 if the amount of TANGO-93 protein or mRNA is above or below a normal level.

For antibody-based kits, the kit may comprise, for example: (1) a first antibody (e.g., attached to a solid support) which binds to TANGO-93 protein; and, optionally, (2) a second, different antibody which binds to TANGO-93 protein or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit may comprise, for example: (1) an oligonucleotide, e.g., a detectably labelled oligonucleotide, which hybridizes to a TANGO-93 nucleic acid sequence or (2) a pair of primers useful for amplifying a TANGO-93 nucleic acid molecule;

The kit may also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit may also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit may also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of TANGO-93.

2. Prognostic Assays

The methods described herein can furthermore be utilized as diagnostic or prognostic assays to identify subjects having or at risk of developing a disease or disorder associated with aberrant TANGO-93 expression or activity. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with TANGO-93 protein, nucleic acid expression or activity. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing such a disease or disorder. Thus, the present invention provides a method in which a test sample is obtained from a subject and TANGO-93 protein or nucleic acid (e.g., mRNA, genomic DNA) is detected, wherein the presence of TANGO-93 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant TANGO-93 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant TANGO-93 expression or activity. For example, such methods can be used to determine whether a subject can be effectively treated with a specific agent or class of agents (e.g., agents of a type which decrease TANGO-93 activity). Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant TANGO-93 expression or activity in which a test sample is obtained and TANGO-93 protein or nucleic acid is detected (e.g., wherein the presence of TANGO-93 protein or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant TANGO-93 expression or activity).

The methods of the invention can also be used to detect genetic lesions or mutations in a TANGO-93 gene, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized by aberrant cell proliferation and/or differentiation. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding a TANGO-93-protein, or the mis-expression of the TANGO-93 gene. For example, such genetic lesions or mutations can be detected by ascertaining the existence of at least one of: 1) a deletion of one or more nucleotides from a TANGO-93 gene; 2) an addition of one or more nucleotides to a TANGO-93 gene; 3) a substitution of one or more nucleotides of a TANGO-93 gene; 4) a chromosomal rearrangement of a TANGO-93 gene; 5) an alteration in the

level of a messenger RNA transcript of a TANGO-93 gene; 6) an aberrant modification of a TANGO-93 gene, such as of the methylation pattern of the genomic DNA; 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a TANGO-93 gene; 8) a non-wild type level of a TANGO-93-protein; 9) an allelic loss of a TANGO-93 gene; and 10) an inappropriate post-translational modification of a TANGO-93-protein. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a TANGO-93 gene. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (*see, e.g.*, U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (*see, e.g.*, Landegran et al. (1988) *Science* 241:1077-1080; and Nakazawa et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:360-364), the latter of which can be particularly useful for detecting point mutations in the TANGO-93-gene (*see, e.g.*, Abravaya et al. (1995) *Nucleic Acids Res.* 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a TANGO-93 gene under conditions such that hybridization and amplification of the TANGO-93-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any

of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) *Bio/Technology* 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a TANGO-93 gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (*see, e.g.*, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in TANGO-93 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin et al. (1996) *Human Mutation* 7:244-255; Kozal et al. (1996) *Nature Medicine* 2:753-759). For example, genetic mutations in TANGO-93 can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin et al., *supra*. Briefly, a

first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the TANGO-93 gene and detect mutations by comparing the sequence of the sample TANGO-93 with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) *Proc. Natl. Acad. Sci. USA* 74:560) or Sanger ((1977) *Proc. Natl. Acad. Sci. USA* 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) *Bio/Techniques* 19:448), including sequencing by mass spectrometry (*see, e.g.*, PCT Publication No. WO 94/16101; Cohen et al. (1996) *Adv. Chromatogr.* 36:127-162; and Griffin et al. (1993) *Appl. Biochem. Biotechnol.* 38:147-159).

Other methods for detecting mutations in the TANGO-93 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) *Science* 230:1242). In general, the technique of "mismatch cleavage" entails providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type TANGO-93 sequence with potentially mutant RNA or DNA

obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands. RNA/DNA duplexes can be treated with RNase to digest mismatched regions, and DNA/DNA hybrids can be treated with S1 nuclease to digest mismatched regions. In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. *See, e.g.*, Cotton et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:4397; Saleeba et al. (1992) *Methods Enzymol.* 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in TANGO-93 cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) *Carcinogenesis* 15:1657-1662). According to an exemplary embodiment, a probe based on a TANGO-93 sequence, e.g., a wild-type TANGO-93 sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. *See, e.g.*, U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in TANGO-93 genes. For example, single strand conformation

polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2766; *see also* Cotton (1993) *Mutat. Res.* 285:125-144; Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control TANGO-93 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, and the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) *Trends Genet.* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) *Nature* 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is

placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) *Nature* 324:163); Saiki et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) *Mol. Cell Probes* 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a TANGO-93 gene.

Furthermore, any cell type or tissue, preferably peripheral blood leukocytes, in which TANGO-93 is expressed may be utilized in the prognostic assays described herein.

3. Pharmacogenomics

Agents, or modulators which have a stimulatory or inhibitory effect on TANGO-93 activity (e.g., TANGO-93 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders (e.g., inflammation) associated with aberrant TANGO-93 activity. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of TANGO-93 protein, expression of TANGO-93 nucleic acid, or mutation content of TANGO-93 genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. *See, e.g.*, Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions

can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body are referred to as "altered drug action." Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as "altered drug metabolism". These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of

ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of TANGO-93 protein, expression of TANGO-93 nucleic acid, or mutation content of TANGO-93 genes in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a TANGO-93 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

4. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of TANGO-93 (e.g., the ability to modulate aberrant cell proliferation and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein, to increase TANGO-93 gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting decreased TANGO-93 gene expression, protein levels, or protein activity. Alternatively, the effectiveness of an agent, as determined by a screening assay, to decrease TANGO-93 gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting increased TANGO-93 gene expression, protein levels, or protein activity. In such clinical trials, TANGO-93 expression or activity and preferably, that of other genes that have been implicated in for example, a

cellular proliferation disorder, can be used as a marker of the immune responsiveness of a particular cell.

For example, and not by way of limitation, genes, including TANGO-93, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates TANGO-93 activity (e.g., as identified in a screening assay described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of TANGO-93 and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of TANGO-93 or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of expression of a TANGO-93 protein, mRNA, or genomic DNA in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level of expression or activity of the TANGO-93 protein, mRNA, or genomic DNA

in the post-administration samples; (v) comparing the level of expression or activity of the TANGO-93 protein, mRNA, or genomic DNA in the pre-administration sample with the TANGO-93 protein, mRNA, or genomic DNA in the post administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example, increased administration of the agent may be desirable to increase the expression or activity of TANGO-93 to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of TANGO-93 to lower levels than detected, i.e., to decrease the effectiveness of the agent.

C. Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant TANGO-93 expression or activity, for example, the TANGO-93 molecules of the present invention may be useful in treating a variety of cellular processes including asthma, graft vs-host disease, rheumatoid arthritis, psoriasis, inflammatory bowel disease, septic shock, ulcerative colitis, Crohn's disease, chronic myelogenous leukemia, cancer, liver disease, Hodgkin's disease, osteoarthritis, Lyme disease, cachexia, and autoimmune diseases, e.g., myasthenia gravis, autoimmune diabetes, and systemic lupus erthematosus.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant TANGO-93 expression or activity, by administering to the subject an agent which modulates

TANGO-93 expression or at least one TANGO-93 activity. Subjects at risk for a disease which is caused or contributed to by aberrant TANGO-93 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the TANGO-93 aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of TANGO-93 aberrancy, for example, a TANGO-93 agonist or TANGO-93 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating TANGO-93 expression or activity for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of TANGO-93 protein activity associated with the cell. An agent that modulates TANGO-93 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of a TANGO-93 protein, a peptide, a TANGO-93 peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of TANGO-93 protein. Examples of such stimulatory agents include active TANGO-93 protein and a nucleic acid molecule encoding TANGO-93 that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of TANGO-93 protein. Examples of such inhibitory agents include antisense TANGO-93 nucleic acid molecules and anti-TANGO-93 antibodies. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in*

vivo (e.g, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a TANGO-93 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) TANGO-93 expression or activity. In another embodiment, the method involves administering a TANGO-93 protein or nucleic acid molecule as therapy to compensate for reduced or aberrant TANGO-93 expression or activity.

Stimulation of TANGO-93 activity is desirable in situations in which TANGO-93 is abnormally downregulated and/or in which increased TANGO-93 activity is likely to have a beneficial effect. Conversely, inhibition of TANGO-93 activity is desirable in situations in which TANGO-93 is abnormally upregulated and/or in which decreased TANGO-93 activity is likely to have a beneficial effect.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

EXAMPLES

Example 1: Isolation and Characterization of Murine TANGO-93 cDNA

Motif designed to identify genes having some similarity to IL-1 was used to search public databases. This led to the identification of a single mouse EST that was present in a mouse embryo cDNA library. The corresponding clone (Genbank # W08205) was obtained and sequenced to completion. The mouse TANGO-93 clone

(Figure 2; SEQ ID NO:1) was found to have an open reading frame (ORF) (SEQ ID NO:3), extending form nucleotide 137 to nucleotide 604 encoding a 156 amino acid gene product (SEQ ID NO:2).

Example 2: Isolation and Characterization of Human TANGO-93 cDNA

Human TANGO-93 was identified independently of mouse TANGO-93. A partial sequence of human TANGO-93 was identified through examination of proprietary databases. A full-length TANGO-93 cDNA clone was obtained by screening an adult keratinocyte cDNA library.

The human TANGO-93 clone (Figure 2; SEQ ID NO:4) was found to have an open reading frame (ORF) (SEQ ID NO:6), extending form nucleotide 57 to nucleotide 521 encoding a 155 amino acid gene product (SEQ ID NO:5).

Additional analysis of human TANGO-93 clones provided additional 3' untranslated sequence. This longer cDNA sequence (SEQ ID NO:9) is shown in Figure 5.

Example 3: Distribution of TANGO-93 mRNA in Mouse Tissues

The expression of TANGO-93 in mice was analyzed using *in situ* hybridization. These studies revealed that the expression of murine TANGO-93 is developmentally regulated. At embryonic Day 14.5, no expression of TANGO-93 was observed in any tissue. At embryonic day 15.5, low level expression was observed at the tip of the nose and in the pancreas. At embryonic day 16.5, as well as at postnatal day 1.5, expression of murine TANGO-93 was observed in the skin (most likely squamous epithelium). No hair follicle expression was observed. Northern blot analysis also revealed that a 1.4 kB TANGO-93 mRNA transcript is present at embryonic day 17 in the skin. The Northern data and the *in situ* hybridization data suggest that TANGO-93 may be involved in tissue development.

In situ hybridization revealed that murine TANGO-93 is constitutively expressed in adult tissues, including the esophagus. No expression of murine TANGO-93 was observed in the brain, eye, heart, thymus, spleen, lung, skeletal, muscle/diaphragm, transverse colon, ascending colon, descending colon, small intestine, liver, kidney, bladder, or adrenal gland.

Following the treatment of mice with LPS, the level of expression of TANGO-93 in various murine tissues was determined using the Perkin-Elmer/ABI 7700 Sequence Detection System (Perkin-Elmer, Norwalk, CT). This system employs TaqMan technology. Briefly, TaqMan technology relies on standard RT-PCR with the addition of a third gene-specific oligonucleotide (referred to as a probe) which has a fluorescent dye at its 5' end (typically 6-FAM) and a quenching dye at its 3' end (typically TAMRA). When the fluorescently tagged probe is intact, the fluorescent signal from the 5' dye is quenched. As PCR proceeds, the 5' to 3' nucleolytic activity of taq polymerase digests the labeled probe, producing a free nucleotide labeled with 6-FAM, which is now detected as a fluorescent signal. The PCR cycle at which fluorescence is first released and detected is directly proportional to the starting amount of the gene of interest in the test sample, thus providing a measure of the initial template concentration. Samples can be internally controlled by the addition of a second set of primers/probe specific for a housekeeping gene such as GAPDH which has been labeled with a different fluorescent at its 5' end (typically JOE).

To determine the level of murine TANGO-93 expression in the various tissues of a mice treated with LPS, a primer/probe set was designed using Primer Express software and primary cDNA sequence information. Total RNA was prepared from a series of murine tissues using an RNeasy kit from Qiagen (Valencia, CA). First strand cDNA

was prepared from 1ug total RNA using an oligo dT primer and Superscript II reverse transcriptase (Gibco/BRL, Mountgomery County, Maryland). cDNA obtained from approximately 50 ng total RNA was used in the TaqMan reaction. TANGO-93 expression was observed eight hours after LPS treatment in the liver, heart, and bone marrow. Little or no TANGO-93 expression was observed in the kidney, brain or lung of LPS-treated mice.

Mice which overexpress TANGO-93 were prepared and challenged with LPS. Mice which overexpress TANGO-93 were significantly resistant to LPS challenged compared to control mice.

Mouse TANGO-93 mRNA expression is also upregulated in Th1 but not Th2 cells. The fact that TANGO-93 is expressed by Th1 cells, but not Th2 cells suggests that TANGO-93 may play a regulatory role in inflammatory and autoimmune diseases, e.g., asthma, graft vs-host disease, rheumatoid arthritis, psoriasis, inflammatory bowel disease, septic shock, ulcerative colitis, Crohn's disease, myasthenia gravis, autoimmune diabetes, and systemic lupus erthematosus.

Northern analysis of human TANGO-93 expression revealed expression in placenta, uterus, and skeletal muscle.

Example 4: Characterization of TANGO-93 Proteins

In this example, the predicted amino acid sequence of murine TANGO-93 protein and human TANGO-93 protein are compared to each other (see Figure 3). The murine TANGO-93 protein and human TANGO-93 protein sequences are also compared to amino acid sequences of known human and murine proteins (see Figure 4). In addition, the molecular weight of the human TANGO-93 protein and murine TANGO-93 protein are predicted.

The murine TANGO-93 cDNA (Figure 1; SEQ ID NO:1) isolated as described above, encodes a 156 amino acid

protein (Figure 1; SEQ ID NO:2), and the human TANGO-93 cDNA (Figure 2; SEQ ID NO:4) isolated as described above encodes a 155 amino acid protein (Figure 2; SEQ ID NO:5). As shown in Figure 3, Human TANGO-93 (SEQ ID NO:5) has 91% identity at the amino acid level to murine TANGO-93 (SEQ ID NO:2).

Figure 4 shows an alignment of murine TANGO-93, human TANGO-93, human IL-1ra and murine IL-1ra. Murine TANGO-93 has 50% and 52% identity at the amino acid level with human IL-1ra (SEQ ID NO:7) and murine IL-1ra (SEQ ID NO:8), respectively (see Figure 4). Human TANGO-93 (SEQ ID NO:2) has 53% identity at the amino acid level with human IL-1ra (SEQ ID NO:7) and 51% identity at the amino acid level with murine IL-1ra (see Figure 4).

Both human TANGO-93 and murine TANGO-93 have a predicted molecular weight of approximately 17 kDa, not including post-translational modifications.

Exammple 5: Chromosome Mapping of Human TANGO-93

The human TANGO-93 gene maps to chromosome 2, within the IL-1 cluster.

Example 6: Assay Confirming that TANGO-93 is Secreted

A secretion assay revealed that human TANGO-93 is secreted when expressed in 293T cells. The secretion assay was performed as follows. Approximately 8 x 10^5 293T cells were plated per well in a 6-well plate, and the cells were incubated in growth medium (DMEM, 10% fetal bovine serum, penicillin/strepomycin) at 37°, 5% CO_2 overnight. The 293T cells were transfected with 2 μg of full-length human TANGO-93 inserted in the pMET7 vector/well and 10 μg LipofectAMINE (GIBCO/BRL Cat. #18324-012)/well according to the protocol for GIBCO/BRL LipofectAMINE. The growth medium was replaced 5 hours

later to allow the cells to recover overnight. Next, the medium was removed and each well was gently washed twice with DMEM without methionine and cysteine (ICN Cat. #16-424-54). Next, 1 ml DMEM without methionine and cysteine with 50 μCi Trans-^{35}S (ICN Cat. #51006) was added to each well and the cells were incubated at 37°, 5% CO_2 for the appropriate time period. A 150 μl aliquot of conditioned medium was obtained and 150 μl of 2X SDS sample buffer was added to the aliquot. The sample was heat-inactivated and loaded on a 4-20% SDS-PAGE gel. The gel was fixed and the presence of secreted protein was detected by autoradiography.

Example 7: Preparation of TANGO-93 Fusion Proteins

Recombinant TANGO-93 can be produced in a variety of expression systems. For example, the TANGO-93 peptide can be expressed as a recombinant glutathione-S-transferase (GST) fusion protein in *E. coli* and the fusion protein can be isolated and characterized. Specifically, as described above, TANGO-93 can be fused to GST and this fusion protein can be expressed in *E. coli* strain PEB199. Expression of the GST-TANGO-93 fusion protein in PEB199 can be induced with IPTG. The recombinant fusion protein can be purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, an amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence of the polypeptide encoded by the cDNA insert of the plasmid deposited with the ATCC at Accession Number 98820, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5, or the polypeptide encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820; and

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6 or a complement thereof under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of:

a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, or a complement thereof; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2 or SEQ ID NO:5;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or a complement thereof under stringent conditions; and

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6 or a complement thereof.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or an amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820;

b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:2, SEQ ID NO:5 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:5, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 98820, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6 or a complement thereof under stringent conditions;

comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

13. A method for detecting the presence of a polypeptide of claim 8 in a sample, comprising:

a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and

b) determining whether the compound binds to the polypeptide in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising the steps of:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of:

a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and

b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by directly detecting binding of the test compound to the polypeptide;

b) detection of binding using a competition binding assay;

c) detection of binding using an assay for TANGO-93-mediated signal transduction.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8, comprising:

a) contacting a polypeptide of claim 8 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

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GAATTTGGCCCTCGAGGCCAAGAATTCGGCACGAGGGGAGCCTGCTTTCTACTTAGGTCTCAAATTTTCCAGCCTTGTC   79

                                                M  M  V  L  S                        5
TTTGCCTAAAATTTCCTGCTGTTTATTTCAAAATAGGGTCTACATACTGTGGAGCTC ATG ATG GTT CTG AGT     151

 G  A  L  C  F  R  M  K  D  S  A  L  K  V  L  Y  L  H  N  N                       25
GGG GCA CTA TGC TTC CGA ATG AAG GAT TCA GCC TTG AAG GTA CTG TAT CTG CAC AAT AAC     211

 Q  L  L  A  G  G  L  H  A  E  K  V  I  K  G  E  E  I  S  V                       45
CAG CTG CTG GCT GGA GGA CTG CAC GCA GAG AAG GTC ATT AAA GGT GAG GAG ATC AGT GTT     271

 V  P  N  R  A  L  D  A  S  L  S  P  V  I  L  G  V  Q  G  G                       65
GTC CCA AAT CGG GCA CTG GAT GCC AGT CTG TCC CCT GTC ATC CTG GGC GTT CAA GGA GGA     331

 S  Q  C  L  S  C  G  T  E  K  G  P  I  L  K  L  E  P  V  N                       85
AGC CAG TGC CTA TCT TGT GGG ACA GAG AAA GGG CCA ATT CTG AAA CTT GAG CCA GTG AAC     391

 I  M  E  L  Y  L  G  A  K  E  S  K  S  F  T  F  Y  R  R  D                      105
ATC ATG GAG CTC TAC CTC GGG GCC AAG GAA TCA AAG AGC TTC ACC TTC TAC CGG CGG GAT     451

 M  G  L  T  S  S  F  E  S  A  A  Y  P  G  W  F  L  C  T  S                      125
ATG GGT CTT ACC TCC AGC TTC GAA TCC GCT GCC TAC CCA GGC TGG TTC CTC TGC ACC TCA     511

 P  E  A  D  Q  P  V  R  L  T  Q  I  P  E  D  P  A  W  D  A                      145
CCG GAA GCT GAC CAG CCT GTC AGG CTC ACT CAG ATC CCT GAG GAC CCC GCC TGG GAT GCT     571

 P  I  T  D  F  Y  F  Q  Q  C  D  *                                               156
CCC ATC ACA GAC TTC TAC TTT CAG CAG TGT GAC TAG                                    607
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FIG. 1A

GGCTGCGTGGTCCCCAAAACTCCATAAGCAGAGGCAGTGGCGGCTCCTGATAGAGGATAGAGACAGAG 686

GAGCTCCACAGTAGGTGGCTTACTCCTCTCCTTCCCTACTGGACTCCCGCTTCTGACCTAAGGCACACAGACACTCTCT 765

TCTCCTGCATCCCAGTGCTGGTAAATCTTCTGGTATTTGGAGCTCAATGTGTAGATTCTTTCAGATTGGATGGTACTAC 844

CTCTGGTGTGGAACCCAATAGAAACCACGTAGGACCAACAAAGAGCAACATAAAAGATTCTTGGGTGAAGAAGAGGTGG 923

GAACTGTTCATACATAGTAAGATCTGACACAGTACCTCAGAAGTCCTGCCATTCCTTATGTTCTGGAGAAAGTGGAGGG 1002

GGGGTCACCAAGACTTTCTCTGGCTGGGCCCTTTCCCTCAACCTTTCTGACATCTGCAGCCTCTCTCATTCTTGC 1081

CTTCATTCTCTGGCCCTGAACCGAGAGGGTGATATCAGGATAGCTGACAGAAGATGACCAGGCACACTGTCCTGGTTTG 1160

AAACCAGAGGGGACAATAAAAAACCCTGATTCTGGTCTCTACTCACATAAAAAGAAGCTTGTGAACATTAAGTGGGAAG 1239

AGATTGCTACTAAATAACATACCTTGGAATTTCATCTTAATTAAAATATACTTCTCTATATTATATTTTAAAAAAAA 1318

AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACATGCGGCCG 1360

FIG. 1B

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                                                                  M   V   L   S   G     5
    GTCGACCCACGCGTCCGGAACATTCTGAGGGGAGTCTACACCCTGTGGAGCTCAAG ATG GTC CTG AGT GGG    71

  A   L   C   F   R   M   K   D   S   A   L   K   V   L   Y   L   H   N   N   Q    25
GCG CTG TGC TTC CGA ATG AAG GAC TCG GCA TTG AAG GTG CTT TAT CTG CAT AAT AAC CAG   131

  L   L   A   G   G   L   H   A   G   K   V   I   K   G   E   E   I   S   V   V    45
CTT CTA GCT GGA GGG CTG CAT GCA GGG AAG GTC ATT AAA GGT GAA GAG ATC AGC GTG GTC   191

  P   N   R   W   L   D   A   S   L   S   P   V   I   L   G   V   Q   G   G   S    65
CCC AAT CGG TGG CTG GAT GCC AGC CTG TCC CCC GTC ATC CTG GGT GTC CAG GGT GGA AGC   251

  Q   C   L   S   C   G   V   G   Q   E   P   T   L   T   L   E   P   V   N   I    85
CAG TGC CTG TCA TGT GGG GTG GGG CAG GAG CCG ACT CTA ACA CTA GAG CCA GTG AAC ATC   311

  M   E   L   Y   L   G   K   K   S   E   S   F   T   F   Y   R   R   D   M   105
ATG GAG CTC TAT CTT GGT GCC AAG GAA TCC AAG AGC TTC ACC TTC TAC CGG CGG GAC ATG   371

  G   L   T   S   S   F   E   S   A   K   Y   P   G   W   F   L   C   T   V   P   125
GGG CTC ACC TCC AGC TTC GAG TCG GCT GCC TAC CCG GGC TGG TTC CTG TGC ACG GTG CCT   431

  E   A   D   Q   P   V   R   L   T   Q   L   P   E   N   G   G   W   N   A   P   145
GAA GCC GAT CAG CCT GTC AGA CTC ACC CAG CTT CCC GAG AAT GGT GGC TGG AAT GCC CCC   491

  I   T   D   F   Y   F   Q   Q   C   D   *                                     155
ATC ACA GAC TTC TAC TTC CAG CAG TGT GAC TAG                                     524
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FIG. 2A

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GGCAACGTGCCCCCCAGAACTCCCTGGGCAGAGCCAGCTCGGGTGAGGGGTGAGTGGAGGAGACCCATGGCGGACAATC  603

ACTCTCTCTGCTCTCAGGACCCCCACGTCTGACTTAGTGGGCACCTGACCACTTTGTCTTCTGGTTCCCAGTTTGGATA  682

AATTCTGAGATTTGGAGCTCAGTCCACGGTCCTCCCCCCACTGGATGGTGCTACTGCTGTGTGGAACCTTGTAAAAACCATG  761

TGGGGTAAACTGGGAATAACATGAAAAGATTTCTGTGGGGGTGGGGGAGTGGTGGGAATCATTCCTGCTTAATG  840

GTAACTGACAAGTGTTACCCTGAGCCCCCGCAGGCCAACCCATCCCCAGTTGAGCCTTATAGGGTCAGTAGCTCTCCACA  919

TGAAGTCCTGTCACTCACCACACTGTGCAGGAGAGGGAGGTGGTCATAGAGTCAGGGATCTATGGCCCTTGGCCCAGCCCC  998

ACCCCCTTCCCTTTAATCCTGCCACTGTCATATGCTACCTTTCCTATCTCTTCCCTCATCATCTTGTTGTGGGCATGAG  1077

GAGGTGGTGATGTCAGAAGAAATGGCTCGAGCTCAGAAGATAAAAGATAAGTAGGGTATGCTGATCCTCTTTTAAAAAC  1156

CCAAGATACAATCAAAATCCCAGATGCTGGTCTCTATTCCCATGAAAAAGTGCTCATGACATATTGAGAAGACCTACTT  1235

ACAAAGTGGCATATATTGCAATTTATTTAATTAAAGATACCTATTTATATATTTCTTTATAAAAAAAAAAAAAAAAG  1314

GGCGGCCGC  1323
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FIG. 2B

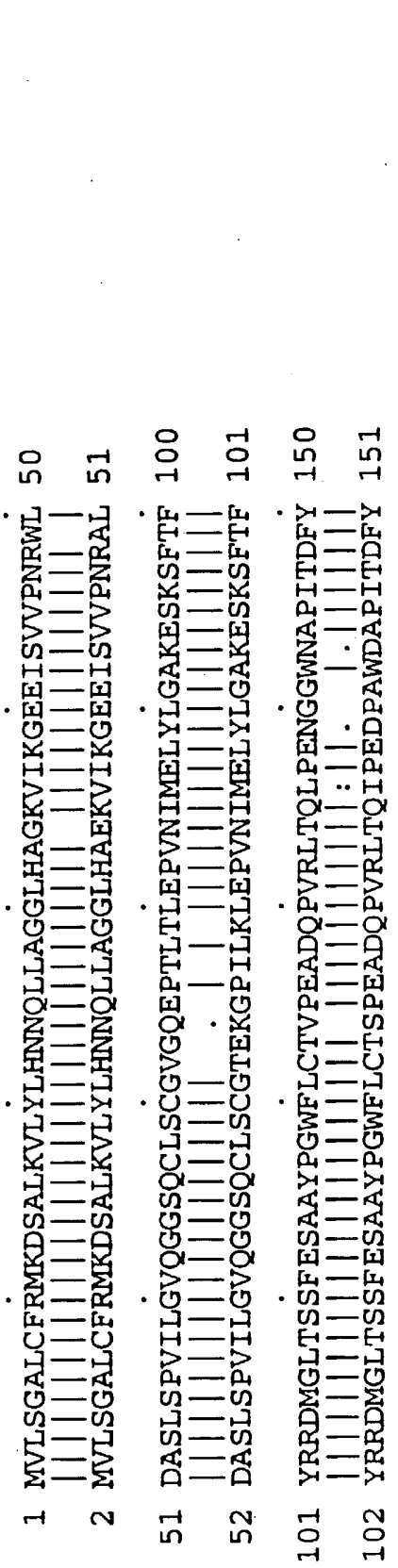
1 MVLSGALCFRMKDSALKVLYLHNNQLLAGGLHAGKVIKGEEISVVPNRWL 50
2 MVLSGALCFRMKDSALKVLYLHNNQLLAGGLHAEKVIKGEEISVVPNRAL 51
51 DASLSPVILGVQGGSQCLSCGVGQEPTLTLEPVNIMELYLGAKESKSFTF 100
52 DASLSPVILGVQGGSQCLSCGTEKGPILKLEPVNIMELYLGAKESKSFTF 101
101 YRRDMGLTSSFESAAYPGWFLCTVPEADQPVRLTQLPENGGWNAPITDFY 150
102 YRRDMGLTSSFESAAYPGWFLCTSPEADQPVRLTQIPEDPAWDAPITDFY 151
151 FQQCD 155
152 FQQCD 156

FIG. 3

```
                                                                  50
1           ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~M MVLSGALCFR MKDSALKVLY
      mt93  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ MVLSGALCFR MKDSALKVLY
      ht93  ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ MVLSGALCFR MKDSALKVLY
illra-human MEICRGLRSH LITLLL.FLF HSETICRPSG RKSSKMQAFR IWDVNQKTFY
illra-mouse MEICWGPYSH LISLLLILLF HSEAACRPSG KRPCKMQAFR IWDTNQKTFY
                                                                  100
51          LHNNQLLAGG LHAEKVIKGE EISVVPNRAL DASLSPVILG VQGGSQCLSC
      mt93  LHNNQLLAGG LHAGKVIKGE EISVVPNRWL DASLSPVILG VQGGSQCLSC
      ht93  LRNNQLVAGY LQGPNVNLEE KIDVVP.... .IEPHALFLG IHGGKMCLSC
illra-human LRNNQLIAGY LQGPNIKLEE KIDMVP.... .IDLHSVFLG IHGGKLCLSC
illra-mouse
                                                                  150
101         G.TEKGPILK LEPVNIMELY LGAKESKSFT FYRRDMGLTS SFESAAYPGW
      mt93  G.VGQEPTLT LEPVNIMELY LGAKESKSFT FYRRDMGLTS SFESAAYPGW
      ht93  VKSGDETRLQ LEAVNITDLS ENRKQDKRFA FIRSDSGPTT SFESAACPGW
illra-human AKSGDDIKLQ LEEVNITDLS KNKEEDKRFT FIRSEKGPTT SFESAACPGW
illra-mouse
                                                                  186
151         FLCTSPEADQ PVRLTQIPED PAWDAPITDF YFQQCD
      mt93  FLCTVPEADQ PVRLTQLPEN GGWNAPITDF YFQQCD
      ht93  FLCTAMEADQ PVSLTNMPDE G...VMVTKF YFQEDE
illra-human FLCTTLEADR PVSLTNTPEE P...LIVTKF YFQEDQ
illra-mouse
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FIG. 4

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                                                                M   V   L   S      4
CCACAGCTCCCGCCAGGAGAAAGGAACATTCTGAGGGGAGTCTACACCCTGTGGAGCTCAAG ATG GTC CTG AGT    74

 G   A   L   C   F   R   M   K   D   S   A   L   K   V   L   Y   L   H   N   N   24
GGG GCG CTG TGC TTC CGA ATG AAG GAC TCG GCA TTG AAG GTG CTT TAT CTG CAT AAT AAC  134

 Q   L   L   A   G   G   L   H   A   G   K   V   I   K   G   E   E   I   S   V   44
CAG CTT CTA GCT GGA GGG CTG CAT GCA GGG AAG GTC ATT AAA GGT GAA GAG ATC AGC GTG  194

 V   P   N   R   W   L   D   A   S   L   S   P   V   I   L   G   V   Q   G   G   64
GTC CCC AAT CGG TGG CTG GAT GCC AGC CTG TCC CCC GTC ATC CTG GGT GTC CAG GGT GGA  254

 S   Q   C   L   S   C   G   V   G   Q   E   P   T   L   T   L   E   P   V   N   84
AGC CAG TGC CTG TCA TGT GGG GTG GGG CAG GAG CCG ACT CTA ACA CTA GAG CCA GTG AAC  314

 I   M   E   L   Y   L   G   A   K   E   S   K   S   F   T   F   Y   R   R   D  104
ATC ATG GAG CTC TAT CTT GGT GCC AAG GAA TCC AAG AGC TTC ACC TTC TAC CGG CGG GAC  374

 M   G   L   T   S   S   F   E   S   A   A   Y   P   G   W   F   L   C   T   V  124
ATG GGG CTC ACC TCC AGC TTC GAG TCG GCT GCC TAC CCG GGC TGG TTC CTG TGC ACG GTG  434

 P   E   A   D   Q   P   V   R   L   T   Q   L   P   E   N   G   G   W   N   A  144
CCT GAA GCC GAT CAG CCT GTC AGA CTC ACC CAG CTT CCC GAG AAT GGT GGC TGG AAT GCC  494

 P   I   T   D   F   Y   F   Q   Q   C   D   *                               156
CCC ATC ACA GAC TTC TAC TTC CAG CAG TGT GAC TAG                               530

GGCAACGTGCCCCCCAGAACTCCCTGGGCAGAGCCAGCTCGGGTGAGGGGTGAGTGGAGGAGACCCATGGCGGACAATC  609

ACTCTCTCTGCTCTCAGGACCCCCACGTCTGACTTAGTGGGCACCTGACCACTTTGTCTTCTGGTTCCCAGTTTGGATA  688

AATTCTGAGATTTGGAGCTCAGTCCACGGTCCTCCCCCACTGGATGGTGCTACTGCTGTGGAACCTTGTAAAAACCATG  767

TGGGGTAAACTGGGAATAACATGAAAAGATTTCTGTGGGGGTGGGGTGGGGGAGTGGTGGGAATCATTCCTGCTTAATG  846

GTAACTGACAAGTGTTACCCTGAGCCCCGCAGGCCAACCCATCCCCAGTTGAGCCTTATAGGGTCAGTAGCTCTCCACA  925

TGAAGTCCTGTCACTCACCACTGTGCAGGAGAGGGAGGTGGTCATAGAGTCAGGGATCTATGGCCCTTGGCCCAGCCCC 1004

ACCCCCTTCCCTTTAATCCTGCCACTGTCATATGCTACCTTTCCTATCTCTTCCCTCATCATCTTGTTGTGGGCATGAG 1083
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FIG. 5A

GAGGTGGTGATGTCAGAAGAAATGGCTCGAGCTCAGAAGATAAAAGATAAGTAGGGTATGCTGATCCTCTTTTAAAAAC 1162

CCAAGATACAATCAAAATCCCAGATGCTGGTCTCTATTCCCATGAAAAAGTGCTCATGACATATTGAGAAGACCTACTT 1241

ACAAAGTGGCATATATTGCAATTTATTTTAATTAAAAGATACCTATTTATATATTTCTTTATAGAAAAAAGTCTGGAAG 1320

AGTTTACTTCAATTGTAGCAATGTCAGGGTGGTGGCAGTATAGGTGATTTTTCTTTTAATTCTGTTAATTTATCTGTAT 1399

TTCCTAATTTTTCTACAATGAAGATGAATTCCTTGTATAAAAATAAGAAAAGAAATTAATCTTGACGTAAGCAGAGCAG 1478

ACATCATCTCTGATTGTCCTCAGCCTCCACTTCCCCAGAGTAAATTCAAATTGAATCGAGCTCTGCTGCTCTGGTTGGT 1557

TGTAGTAGTGATCAGGAAACAGATCTCAGCAAAGCCACTGAGGAGGAGGCTGTGCTGAGTTTGTGTGGCTGGAATCTCT 1636

GGGTAAGGAACTTAAAGAACAAAAATCATCTGGTAATTCTTTCCTAGAAGGATCACAGCCCCTGGGATTCCAAGGCATT 1715

GGATCCAGTCTCTAAGAAGGCTGCTGTACTGGTTGAATTGTGTCCCCCTCAAATTCACATCCTTCTTGGAATCTCAGTC 1794

TGTGAGTTTATTTGGAGATAAGGTCTCTGCAGATGTAGTTAGTTAAGACAAGGTCATGCTGGATGAAGGTAGACCTAAA 1873

TTCAATATGACTGGTTTCCTTGTATGAAAAGGAGAGGACACAGAGACAGAGGAGACGCGGGGAAGACTATGTAAAGATG 1952

AAGGCAGAGATCGGAGTTTTGCAGCCACAAGCTAAGAAACACCAAGGATTGTGGCAACCATCAGAAGCTTGGAAGAGGC 2031

AAAGAAGAATTCTTCCCAAGAATAAATTTCGGCTGTTTTAAGCCACCAAGGATAATTGGTTACAGCAGCTCTAGGAAAC 2110

TAATACAGCTGCTAAAATGATCCCTGTCTCCTCGTGTTTACATTCTGTGTGTGTCCCCTCCCACAATGTACCAAAGTTG 2189

TCTTTGTGACCAATAGAATATGGCAGAAGTGATGGCATGCCACTTCCAAGATTAGGTTATAAAAGACACTGCAGCTTCT 2268

ACTTGAGCCCTCTCTCTCTGCCACCCACCGCCCCCAATCTATCTTGGCTCACTCGCTCTGGGGGAAGCTAGCTGCCATG 2347

CTATGAGCAGGCCTATAAAGAGACTTACGTGGTAAAAAATGAAGTCTCCTGCCCACAGCCACATTAGTGAACCTAGAAG 2426

CAGAGACTCTGTGAGATAATCGATGTTTGTTGTTTTAAGTTGCTCAGTTTTGGTCTAACTTGTT 2490

FIG. 5B